

Decreased NK-Cell Cytotoxicity after Short Flights on the Space Shuttle

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Abstract

Cytotoxic activity of natural killer (NK) cells and cell surface marker expression of peripheral blood mononuclear cells (PBMCs) isolated from 11 U.S. astronauts on two different missions were determined before and after 9 or 10 days of spaceflight aboard the space shuttle. Blood samples were collected 10 and 3 days before launch, within 3 hours after landing, and 3 days after landing. All PBMC preparations were cryopreserved and analyzed simultaneously in a 4-hour cytotoxicity ^{51}Cr -release assay using NK-sensitive K-562 target cells. Compared to preflight values, NK-cell cytotoxicity (corrected for lymphopenia observed on landing day) was significantly decreased at landing ($P < 0.0125$). It then apparently began to recover and approached preflight values by 3 days after landing. Consistent with decreased NK-cell cytotoxicity, significant increases from preflight values were found in plasma adrenocorticotrophic hormone at landing. Plasma and urinary cortisol levels did not change significantly from preflight values. Expression of major lymphocyte surface markers (CD3, CD4, CD8, CD14, CD16, CD56), determined by flow cytometric analysis, revealed no consistent phenotypic changes in relative percent of NK or other lymphoid cells after 10 days of spaceflight.

Keywords: space immunology; microgravity; cytotoxicity; NK cells

The human immune response changes during short and long spaceflights (1, 7, 18, 19, 21, 22, 24, 25, 27). Missions of 10 days or less have been associated with reductions in absolute numbers of lymphocytes, eosinophils, and natural killer (NK) cells,¹ and elevations in the total number of neutrophils in peripheral blood (19, 25). In other studies, reduced lymphocyte mitogenic response (1, 24), diminished delayed-type hypersensitivity (25), changes in CD4⁺/CD8⁺ ratios, and reduced production of IL-2 and interferon (IFN)- γ have been observed. Russian investigators have reported significant decreases in the NK-cell cytotoxicity index in 17 of 33 cosmonauts after spaceflight of 60 to 366 days (7, 8). The index returned to preflight levels in approximately half of these cosmonauts by 14 days after landing. Prior to this report, no information on NK-cell function after short spaceflights has been available.

As NASA prepares for long-term habitation of the International Space Station, establishment of a lunar outpost, and human exploration of Mars, it is important to understand the effect of spaceflight on the immune system. NK-cell function is an important element of the immune system and essential in assessing health effects of long spaceflight missions. Until now, NK-cell cytotoxicity of U.S. astronauts has not been measured.

In this study, we assessed the effect of spaceflight on spontaneous NK-cell cytotoxicity and on expression of selected surface markers on peripheral blood mononuclear cells (PBMCs). Blood was collected from 11 members of 2 space shuttle crews before and after flight, and PBMC fractions were cryopreserved for later analysis with a ⁵¹Cr-release assay using K-562 target cells (9). Cytotoxic activity in crewmembers' blood was significantly reduced on landing day compared to preflight values.

Materials and Methods

Collection and cryopreservation of blood cells. A total of 11 crewmembers (9 males and 2 females) participated in this study, which was approved by the Johnson Space Center Institutional Review Board. Crewmembers 1–6 were from mission 1 (10 days) and crewmembers 7–11 were from mission 2 (9 days). Blood samples (10 ml) were collected from each crewmember into EDTA-containing vacutainers at 4 time points: 10 and 3 days before launch (L–10 and L–3), within 3 hours after landing (R+0), and 3 days after landing (R+3). Immediately after collection, the blood was diluted 1:1 with normal saline, underlain with 15 ml of Ficoll Hypaque (Pharmacia, Uppsala, Sweden), and centrifuged for 30 min at 400 g. The mononuclear layer was collected and washed with Hank's balanced salt solution without Ca^{+2} or Mg^{+2} (Biowhittaker, Walkersville, MD). Cells were counted, and resuspended in 1 ml of freezing medium consisting of 90% pooled human blood group AB serum (Biowhittaker, Walkersville, MD) and 10% dimethylsulfoxide (Sigma Chemical Co., St. Louis, MO). These cells were then cryopreserved by placing them in a -70°C bath of 100% ethanol, then transferred the next day to liquid nitrogen, where they were stored until analyzed for cytotoxicity.

^{51}Cr -release assay. Cytolytic activity of NK cells was measured by the standard assay of Lagoo-Deenadayalan et al. (9). Briefly, target K-562 cells were labeled with $\text{Na}_2^{51}\text{CrO}_4$ (DuPont, Boston, MA), washed, and incubated for 4 hours with effector cells at effector-to-target (E:T) ratios of 100:1, 50:1, and 25:1. Percent lysis by the NK cells at each measurement point was calculated as (experiment cpm minus spontaneous cpm / maximum cpm minus spontaneous cpm) $\times 100$. To account for potential changes in lymphocyte number at the measurement times, the NK-cell cytotoxicity per 10^3 lymphocytes was normalized in the following way:

% NK-cell cytotoxicity per 10^3 lymphocytes = (% lysis by NK cells/ number of lymphocytes per mm^3 of blood) $\times 1000$.

Absolute numbers of white blood cells (WBCs) and percent lymphocytes also were determined with a Coulter MAXM (Coulter Electronics Inc., Miami, FL) at each measurement point for normalization of cytotoxic function.

Endocrinology. Plasma and urine cortisol and plasma adrenocorticotrophic hormone (ACTH) were measured by radioimmunoassay (2,16).

Lymphocyte surface markers. PBMCs were resuspended at a concentration of 10^5 cells/100 μl of phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.1% sodium azide, and the aliquots were incubated for 30 min at 4 °C with each of the following monoclonal antibodies: anti-CD3-FITC¹, anti-CD56-PE, anti-CD4-FITC, anti-CD8-PE, anti-CD14-FITC, and anti-CD16-PE (Becton Dickinson, San Jose, CA). Mouse IgG1-FITC and mouse IgG1-PE (Becton Dickinson) were used as isotype controls. After staining, the cells were washed with PBS, fixed in 0.5 ml of 1% paraformaldehyde, and analyzed on a FACScan (Becton Dickinson) flow cytometer. Ten thousand events were collected for each sample and the gates were set on the lymphocyte population based upon forward and side scatter. The results were expressed as the percent of gated cells positive for each surface marker.

Statistical analysis. Although three different effector:target ratios (100:1, 50:1 and 25:1) were used, data from only one effector:target ratio (100:1) were analyzed statistically. The same trends of changes in percent cytotoxicity were observed at both lower effector:target ratios. Statistical analyses were performed on the data for the percent cytotoxicity and normalized percent cytotoxicity of NK cells from all 11 crewmembers measured at 4 time points: 2 preflight

days (L-10 and L-3) and 2 postflight days (R+0 and R+3). Student's paired t-test and analysis of variance (ANOVA) were used to test for differences in percent cytotoxicity between the various preflight and postflight time points. Differences were considered statistically significant if $P < 0.05$.

Results

The percent cytotoxicity of NK cells from 11 crewmembers on two missions is shown in Fig. 1, along with urine and plasma levels of cortisol and plasma levels of ACTH from the same crewmembers. The percent cytotoxicity was lower on landing day (R+0) than on either preflight sampling day (L-3: $P < 0.0125$, L-10: $P < 0.06$) Postflight plasma and urine cortisol levels were not significantly different from preflight values. However, ACTH levels were significantly increased ($P < 0.001$) at landing to 66.0 (mean) ± 15.29 (standard error of the mean) $\mu\text{g/ml}$ from preflight values of 28.54 ± 13.95 $\mu\text{g/ml}$ at L-10 and 47.63 ± 19.50 $\mu\text{g/ml}$ at L-3. ACTH levels returned to L-3 values by three days after landing (48.09 ± 17.02 $\mu\text{g/ml}$ at R+3).

Individual patterns of change in percent cytotoxicity for mission 1 and mission 2 are shown in Fig. 2 (a and b). During the 7-day period from L-10 to L-3, the percent cytotoxicity values increased for 7 of the 11 crewmembers ($P \leq 0.05$) and decreased for the other 4. From R+0 to R+3, percent cytotoxicity also increased significantly for 7 of the 11 crewmembers ($P \leq 0.05$) and decreased for the remaining 4 crewmembers. A comparison of preflight and postflight data showed that percent cytotoxicity on landing day was less than on either L-10 or L-3 for crewmembers 2, 4, 5, 6, 7, 8, 9, 10, and 11 and less than L-3 values (but not L-10 values) for

crewmembers 1 and 3. Individually, this decrease was statistically significant ($P \leq 0.05$) for all but one crewmember (crewmember 9). One prominent difference between missions 1 and 2 was found: R+3 cytotoxicity values increased from the R+0 values in all 6 crewmembers on mission 1, and 5 of these 6 crewmembers showed a rebound in percent cytotoxicity to near preflight values. However, 4 of the 5 crewmembers from mission 2 showed a drop in percent cytotoxicity ($P \leq 0.05$) from R+0 to R+3.

We also examined cytotoxicity in terms of lymphocyte numbers. Although the total numbers of WBCs per mm^3 of blood for each crewmember did not change over time, the percentage of lymphocytes was reduced by >5 percentage points on landing day for 8 of 11 crewmembers (Table 1). To minimize these changes, we normalized the percent cytotoxicity of the PBMC population by using percent lymphocyte values at each measurement time to reflect equivalents of 10^3 lymphocytes at those times (Fig. 3). When all 11 crewmembers were analyzed as a group, normalized cytotoxicity values were significantly lower on R+0 than on either L-10 or L-3 ($P \leq 0.05$) (Table 2). When the results from individual crewmembers were analyzed, normalized percent cytotoxicity values were significantly lower on R+0 than on either L-10 or L-3 or both, in 9 of 11 crewmembers (crewmembers 1, 2, 4, 5, 6, 7, 8, 10, and 11) ($P \leq 0.05$). On R+3, the normalized percent cytotoxicity of 5 of 11 crewmembers (1, 2, 3, 4, and 6) had returned to or exceeded preflight (L-10 or L-3) values (Fig. 3). Small differences were noted between percent cytotoxicity and normalized percent cytotoxicity values (see crewmembers 3 and 5 in Figs. 2a and 3a).

The results of the cytotoxicity studies of crewmembers as a group and as individuals by mission are summarized in Table 2. The interpretation was the same, whether the percent

cytotoxicity or normalized percent cytotoxicity was used. When the 11 crewmembers were considered as one random sample group or as individuals (see Table 2), the percent cytotoxicity (normalized or not-normalized) on landing day (R+0) was significantly lower than on either preflight sampling day (L-3: $P < 0.0125$, L-10: $P < 0.06$). The observed differences in cytotoxic activity for the group of 11 crewmembers at L-10 vs. L-3, or at R+0 vs. R+3, were not statistically significant. However, individually some did show significant differences (Table 2).

Flow cytometry was used to assess phenotypic changes in expression of CD3, CD4, CD8, CD14, CD16, and CD56 on PBMCs. No significant changes from preflight values were evident in the NK markers CD56 and CD16 after mission 1 (data not shown). Except for crewmember 2, CD14⁺ (LeuM3⁺) cells (monocytes) had decreased at L-3, and had returned to L-10 values on R+0 and R+3. The total percentage of CD3⁺ T cells increased at R+0 (except for crewmember 5, whose T cells decreased at R+0), and by R+3 either returned to preflight values (crewmembers 1, 4, 6) or increased further (crewmembers 2 and 3). In a majority of crewmembers on mission 2, the percentage of CD16⁺ and CD56⁺ cells decreased. The percentage of CD16⁺ cells decreased in 3 crewmembers (crewmembers 8, 9, and 11), and the percentage of CD56⁺ cells decreased (1.5- to 4-fold) in 4 crewmembers (crewmembers 8, 9, 10, and 11).

Discussion

This is the first study of NK cell function of US astronauts. We undertook this study to determine if NK-cell cytotoxic activity would be decreased after short (< 15 days) spaceflights as it was during long Russian space missions (7, 8). We found decreased NK-cell cytotoxic activity after 9 to 10 days aboard the space shuttle, similar in magnitude to the Russian findings after 60

to 366 days in space. This suggests that NK-cell cytotoxic activity occurs relatively early during spaceflight and recovers to typical preflight values after landing. Similar rapid changes in the cell-mediated immune response have been demonstrated by Taylor and Janney (25).

Alternatively, the decreased NK-cell cytotoxic activity may be a transient response to periods of stress such as launch, inflight emergencies, and landing, and returns to typical values during low-stress periods in space or on the Earth.

In Russian studies of long space missions (60–366 days), the reduced postflight cytotoxic activity levels of some cosmonauts had returned to preflight values by 14 days after landing, but cytotoxic activity levels of others had not returned to preflight values after 2 months (8). Our results indicate that by 3 days after landing, cytotoxic activity levels of most of the astronauts were returning to preflight values. These two flights showed similar patterns in NK-cell cytotoxicity changes except for the recovery of function after landing. All of the crewmembers on mission 1 showed a recovery by three days after landing, but crewmembers on mission 2 did not. The reason(s) for the differences observed in the recovery of NK-cell cytotoxic activity after the 9 and 10 day missions were not determined. These differences may be due to normal variability between individual crewmembers, differences between the two spaceflights, or a combination of these factors. The durations of the missions differed by only one day, and the physical environments (e.g., temperature and humidity) on the two spacecraft were nearly identical. The composition of the two crews was examined with respect to age, gender, experience (e.g., first-time or repeat flyers), and mission role (e.g., pilot or scientist), and no correlation was found with respect to individual NK-cell function. Two crewmembers performed an extravehicular activity (space walk) on mission 1 with no apparent effect on NK-cell function. Because of the individual differences observed in NK-cell function (and other immune

parameters), each astronaut served as their own control. The cytotoxicity values obtained at L-3 and L-10 days before launch served as preflight baseline values to which postflight values could be compared on an individual basis.

Rykova et al. (17) reported finding a similar decrease in rat NK-cell cytotoxicity during a 14-day space mission using a murine NK-cell target (YAC1). They observed no decrease when K-562 (a human NK-cell target) target cells were used. Decreased NK-cell cytotoxicity in spleen and bone marrow of rats flown aboard the Russian Cosmos biosatellites has also been reported (13).

Phenotypic expression of NK markers CD16 and CD56 did not correlate with the decrease in NK-cell cytotoxic activity observed at landing. In an earlier report (22), 11 astronauts showed considerable variation in phenotypic expression of these leukocyte markers after landing. For example, expression of CD4 was higher in 8 astronauts and lower in the remaining 3, and CD8 was lower in 5 and higher in 5 of the 11 astronauts. Expression of CD16 and CD56 was lower at landing than the preflight values. The current findings showed similar variations in surface marker expression (data not shown).

We controlled variation between assays by cryopreserving PBMCs from both missions and simultaneously assaying them later. This was necessary because the cells were collected over a 23-day period. Letellier et al. (14) and Fujiwara et al. (3) have shown that cryopreservation and thawing does not affect the lymphocyte subset expression of CD2, CD3, CD4, CD8, CD16, CD56, and CD57. Cryopreservation may affect NK or lymphokine-activated killer cell activities (4, 6), and stringent adherence to the thawing technique was critical in preventing loss of activity. Since all the cells used in the study were uniformly cryopreserved and thawed, any effect was common to all cells.

The purpose of this study was to determine the effect of short spaceflights on NK-cell cytotoxicity. A priority for future studies is to understand the cause of the decrease in NK-cell cytotoxic activity observed after short spaceflights. Many physical and psychological stresses are associated with spaceflight (15), and the effects of stress on immune function are well recognized (4). Sometimes these effects are associated with the “stress hormones,” which include cortisol, ACTH, growth hormone, and beta endorphin. Stein and Schluter (20) showed that urinary excretion of IL-6 and cortisol increased significantly only on the first day of spaceflight (after launch) and on landing day. They did not observe additional changes before, during, or after flight. Our data showed no significant change in urinary and plasma cortisol levels before or after the missions, but plasma ACTH was significantly increased at landing. Plasma ACTH levels were higher at landing than before flight for a group of eight other space shuttle crewmembers (11); however, levels were higher on flight day 12 and not on landing day for crewmembers on the Spacelab Life Sciences missions (10). Significantly raised levels of human growth hormone at landing have also been reported (12). Kay et al. (6) have shown that beta endorphins and several analogues (e.g., gamma endorphins) enhance human peripheral blood NK-cell function. Further studies on stress and stress hormones are necessary to better understand decreased NK-cell function after spaceflight.

The mechanisms responsible for the decrease in NK-cell activity are not clear and may be the result of a combination of factors, including the inhibitory effect of glucocorticoids and failure of NK cells to migrate from bone marrow to the periphery (26). The changes reported here in NK-cell activity and in the hormone profile are consistent with stress-induced effects of spaceflight on the neuroendocrine and immune systems.

Acknowledgments

We thank Christine Wogan and Jane Krauhs of Wyle Laboratories, Life Sciences, Systems and Services for editorial comments. Statistical analyses were performed by R. S. Chhikara.

This work was supported in part by the NASA-Mir Program and by a National Research Council Senior Research Associateship to SKM.

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Footnotes

¹Abbreviations used in this paper: ACTH, adrenocorticotrophic hormone; ANOVA, analysis of variance; E:T, effector-to-target; FITC, fluorescein isothiocyanate; NK cells, natural killer cells; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PE, phycoerythrin; WBC, white blood cell.

Figure Legends

Fig. 1. Percent cytotoxicity of NK cells (E:T ratio 100:1) and levels of urine ($\mu\text{g}/24\text{ h}$) and plasma ($\mu\text{g}/\text{dl}$) cortisol and plasma ACTH (pg/ml) from 11 crewmembers on 2 spaceflight missions. Blood samples were collected twice before launch (days L-10 and L-3), on landing day (R+0), and 3 days after landing (R+3). Values are means with standard error of the means.

Fig. 2. Cytotoxicity of NK cells from 6 crewmembers on mission 1 (a) and 5 crewmembers on mission 2 (b). Blood samples were collected twice before launch (days L-10 and L-3), on landing day (R+0), and three days after landing (R+3). Cytotoxicity was assessed with a standard ^{51}Cr -release assay with K-562 cells as a target; data shown are for an E:T ratio of 100:1. Values are means of 3 replicates, with standard errors.

Fig. 3. NK-cell cytotoxicity normalized per 10^3 lymphocytes from 6 crewmembers on mission 1 (a) and 5 crewmembers on mission 2 (b), estimated from a standard ^{51}Cr -release assay with K-562 cells as a target. Blood samples were drawn before flight (days L-10 and L-3), on landing day (R+0), and 3 days after landing (R+3). Values are means of 3 replicates, with standard errors.

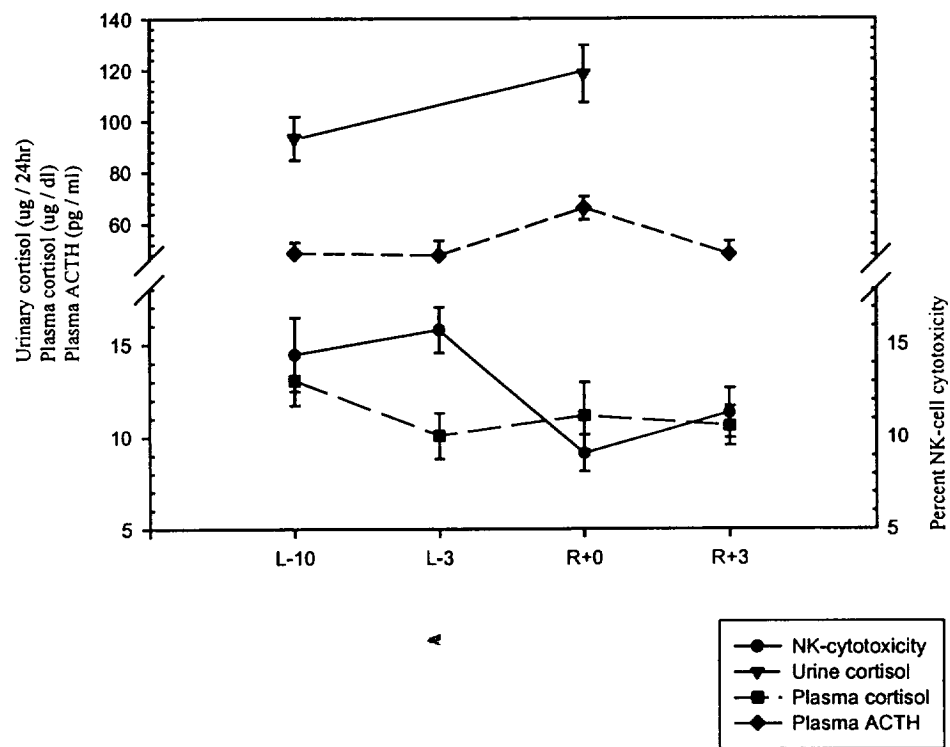


Figure 1

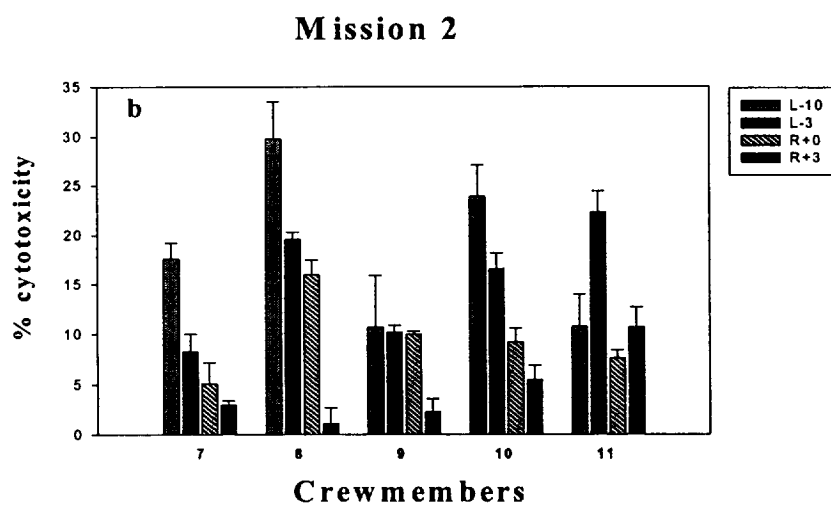
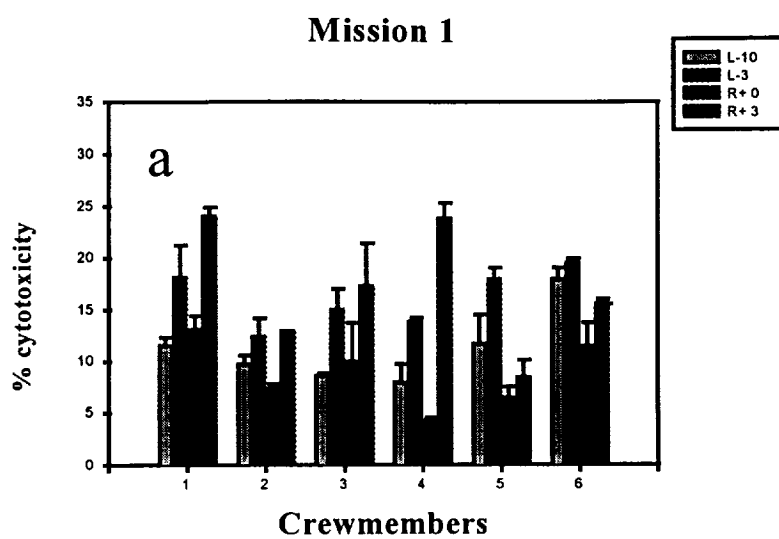
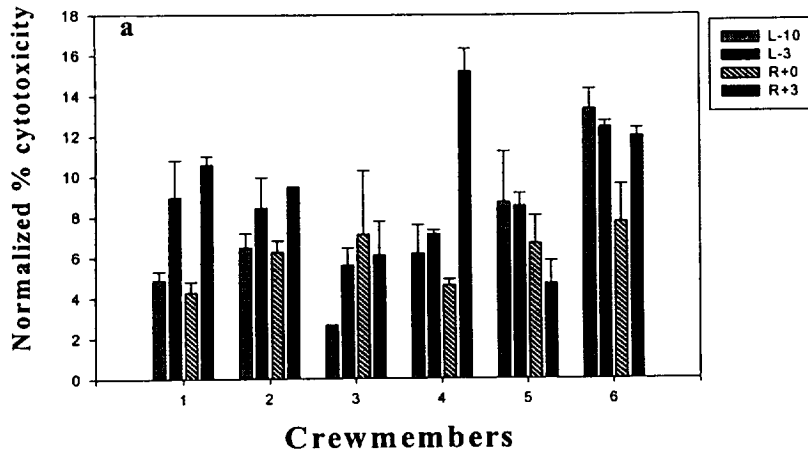


Figure 2

Mission 1



A

Mission 2

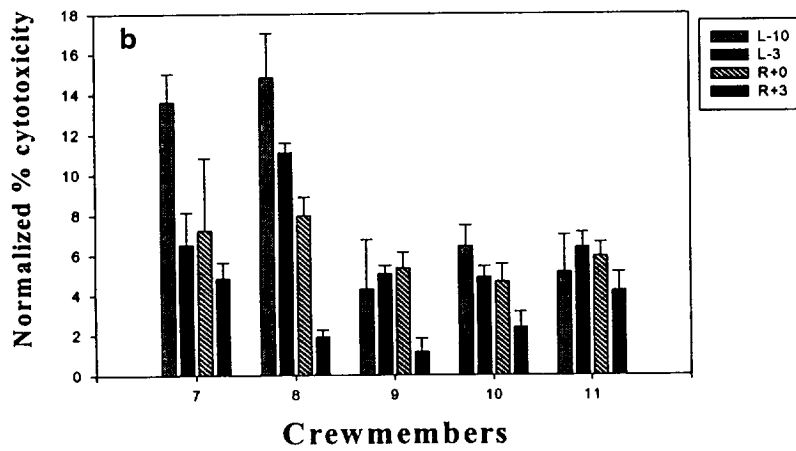


Figure 3

Table 1. *Number of white blood cells (WBCs) and lymphocytes obtained from the peripheral blood of crewmembers on 2 space shuttle missions*

Crew-Member	Day	No. of WBCs/mm ³ blood ($\times 10^3$)	% lymphocytes in WBCs	No. of lymphocytes/mm ³ blood ($\times 10^3$)
1	L-10*	5.9	40	2.4
	L-3	6.5	31	2.1
	R+0	8.3	37	3.1
	R+3	5.4	42	2.3
2	L-10	3.4	44	1.5
	L-3	3.6	41	1.5
	R+0	5.3	22	1.2
	R+3	2.9	47	1.4
3	L-10	7.4	45	3.3
	L-3	6.4	42	2.7
	R+0	7.8	18	1.4
	R+3	6.8	42	2.9
4	L-10	3.9	33	1.3
	L-3	4.4	44	1.9
	R+0	7.6	12	0.9
	R+3	4.9	32	1.6
5	L-10	5.6	24	1.3
	L-3	6.0	35	2.1
	R+0	6.0	16	1.0
	R+3	4.6	39	1.8
6	L-10	4.2	32	1.3
	L-3	5.2	30	1.6
	R+0	4.5	33	1.5
	R+3	4.3	30	1.3
7	L-10	4.3	30	1.3
	L-3	4.7	27	1.3
	R+0	6.4	11	0.7
	R+3	3.9	16	0.6

8	L-10	4.7	43	2.0
	L-3	5.7	31	1.8
	R+0	6.7	30	2.0
	R+3	5.4	24	1.3
9	L-10	5.8	43	2.5
	L-3	5.9	34	2.0
	R+0	9.7	22	2.1
	R+3	5.5	39	2.1
10	L-10	7.0	53	3.7
	L-3	6.8	50	3.4
	R+0	7.6	26	2.0
	R+3	6.6	35	2.3
11	L-10	5.7	37	2.1
	L-3	7.2	48	3.5
	R+0	11.6	11	1.3
	R+3	6.0	42	2.5

*L-x, days before launch; R+y, days after landing

Table 2. Summary of results of statistical analysis of NK-cell activity in blood samples from 11 crewmembers on 2 space shuttle flights

% Cytotoxicity			
Group analysis		Individual analysis	Normalized % cytotoxicity
Group analysis		Individual analysis	Individual analysis
<i>Preflight</i>			
L-3 and L-10 not significantly different from each other.	*L-10 < L-3 7/11 crewmembers Mission 1: 6/6 Mission 2: 1/5	L-3 and L-10 not significantly different from each other.	*L-10 < L-3 5/11 crewmembers Mission 1: 3/6 Mission 2: 2/5
<i>Landing</i>			
*R+0 < preflight values	*R+0 < preflight values 10/11 crewmembers Mission 1: 6/6 Mission 2: 4/5	*R+0 < preflight values	*R+0 < preflight values 9/11 crewmembers Mission 1: 5/6 Mission 2: 4/5
<i>Recovery</i>			
R+0 and R+3 not significantly different from each other.	*R+0 < R+3 7/11 crewmembers Mission 1: 6/6 Mission 2: 1/6	R+0 and R+3 not significantly different from each other.	*R+0 < R+3 5/11 crewmembers Mission 1: 4/6 Mission 2: 0/5

R+3 not significantly different
from preflight values
5/11 crewmembers
Mission 1: 5/6
Mission 2: 0/5

R+3 not significantly different
from preflight values
3/11 crewmembers
Mission 1: 3/6
Mission 2: 0/5

* Statistically significant ($P < 0.05$). L-x, days before launch; R+y, days after landing

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